FONDAZIONE EDMUND MACH

Interreg Alpine Space Priority 3: Liveable Alpine Space. SO3.2: Enhance the protection, the conservation and the ecological connectivity of Alpine Space

Interreg Alpine Space Eco-AlpsWater European Regional Development Fund

Seminario di presentazione del progetto Eco-AlpsWater
Sede ISPRA, Sala del Consiglio Federale, Roma, 16 ottobre 2019

Eco-AlpsWater

Innovative Ecological Assessment and Water Management Strategy for the Protection of Ecosystem Services in Alpine Lakes and Rivers

Microscopia classica:

limiti dell'approccio tradizionale

Nico Salmaso

Coordinatore del progetto Eco-AlpsWater

IASMA Research and Innovation Centre
Fondazione Mach-Istituto Agrario di S. Michele all'Adige
Unità di ricerca Idrobiologia
nico.salmaso@fmach.it – 0461 615323

Premise (1) – that is, where did we start from?

The EU **Water Framework Directive 2000/60/EC** commits European Union member states to achieve "good" qualitative and quantitative status of all water bodies.

The ecological and chemical status of surface waters are assessed according to the following criteria:

- **Biological quality**, based on microalgae, fish, benthic invertebrates, aquatic flora
- **Physical-chemical quality** such as temperature, oxygenation and nutrient conditions
- **Chemical quality**, referring to environmental quality standards for specific pollutants. These standards specify maximum concentrations for specific water pollutants. If even one such concentration is exceeded, the water body will not be classed as having a "good ecological status".
- **Hydromorphological quality**, including river bank structure, river continuity, substrate of the river bed

Premise (2)

In the history and implementation of the Water Framework Directive, most of the results in lakes were focused on the use of microalgae (phytoplankton and microalgae living on substrates – biofilm) as biodindicators.

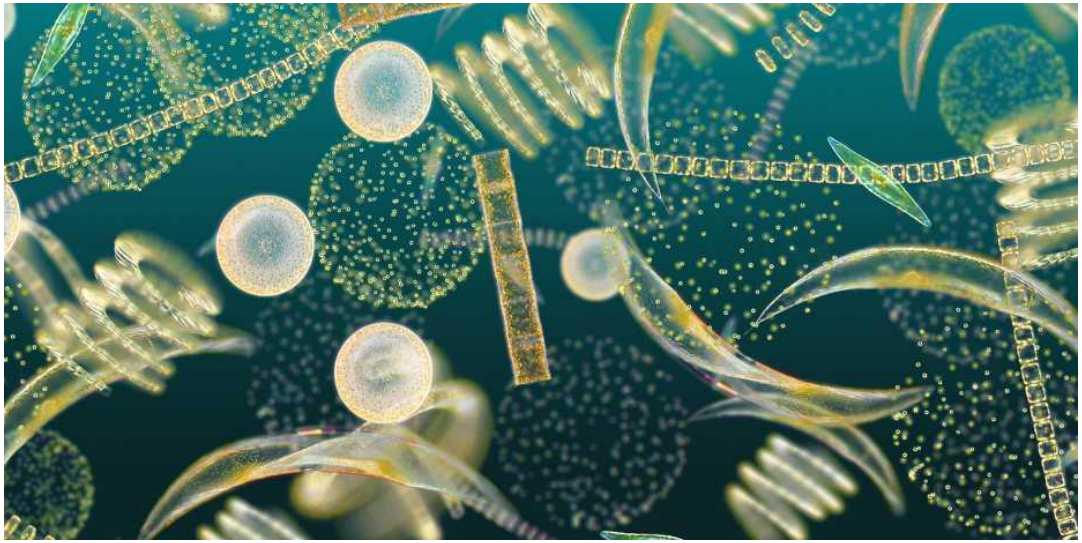

Illustration: www.secchidisk.org

In this regard, biomonitoring involves the use of microalgae to assess ecological status, by quantifying changes in organisms. By evaluating the effects of environment on organisms, changes may be suspected or proved.

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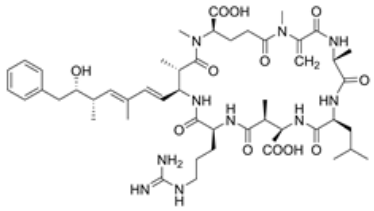
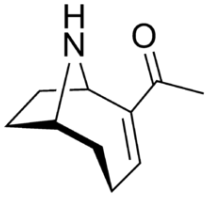
Premise (3) - Importance of a correct identification of microalgae

Cianobatteri: almeno 3 caratteristiche negative principali

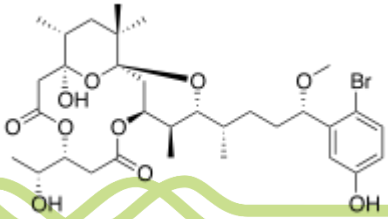
1. Aumentano nei laghi ricchi di nutrienti (tipiche specie eutrofiche)
2. Danno luogo a «fioriture algali»
3. Possono svilupparsi con ceppi tossici, in grado di produrre un'ampia varietà di tossine



Epatotossine e potenziali promotori tumorali

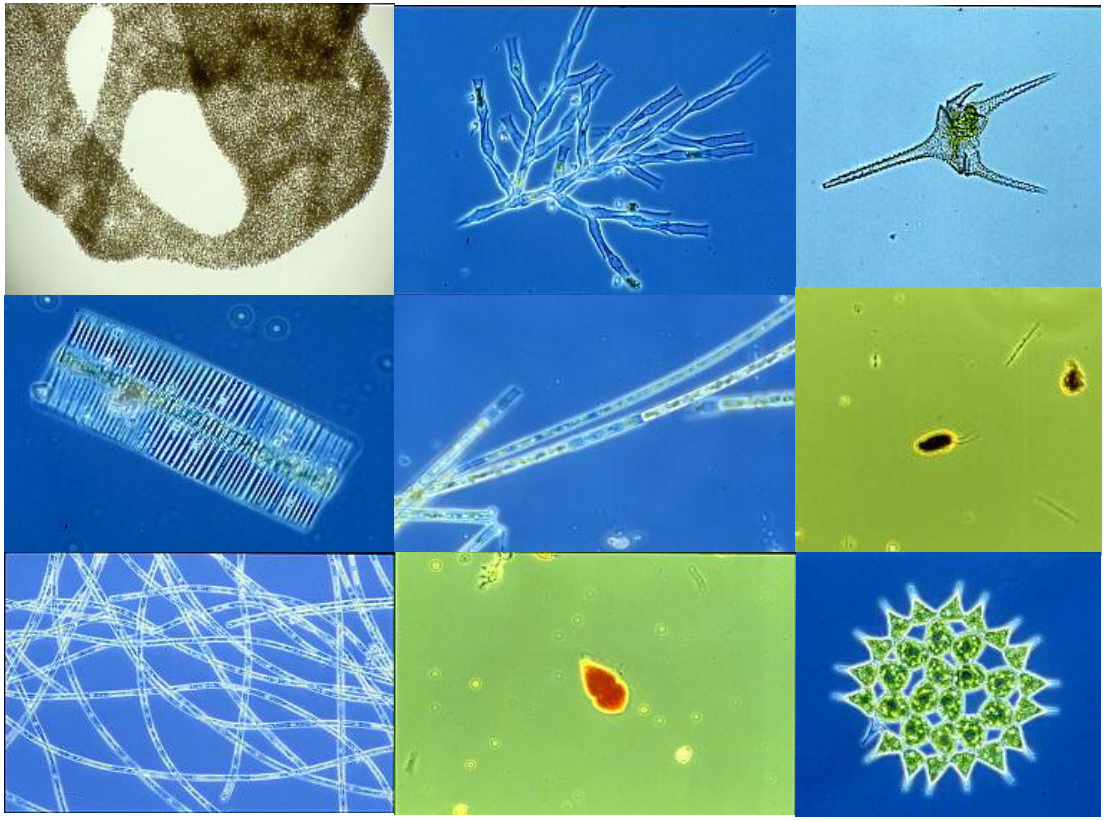


Neurotossine



Dermatotossine (es. Aplisiatossine), irritanti e carcinogenici (prodotte da specie marine)

Microalgae can be identified by optical microscopy



<1 µm – 200 µm
1 µm = 1/1000 mm

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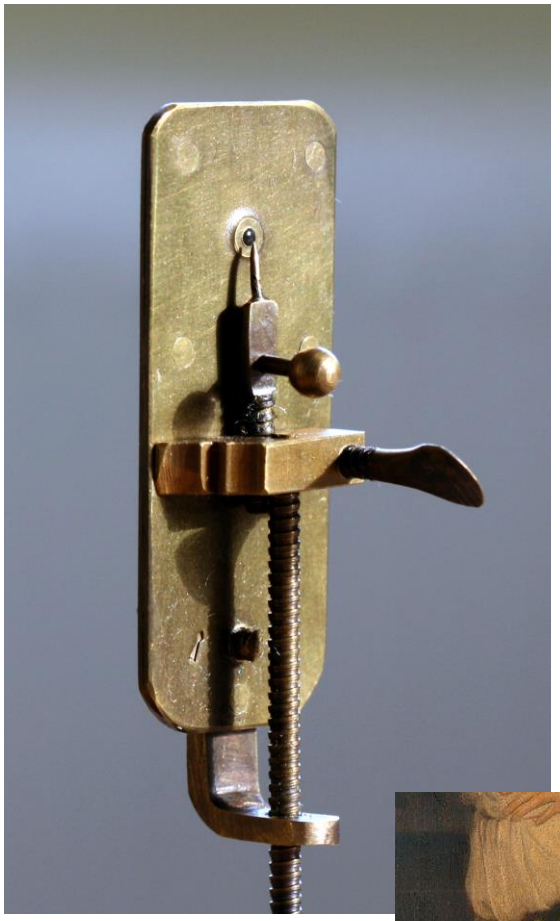
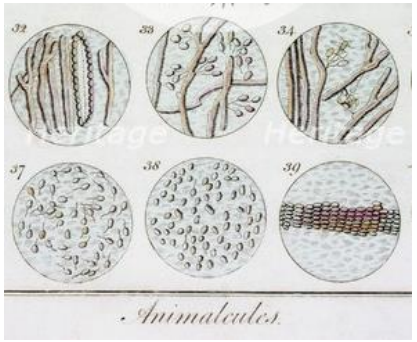
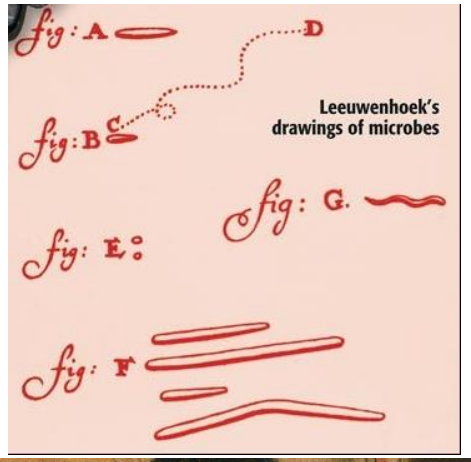
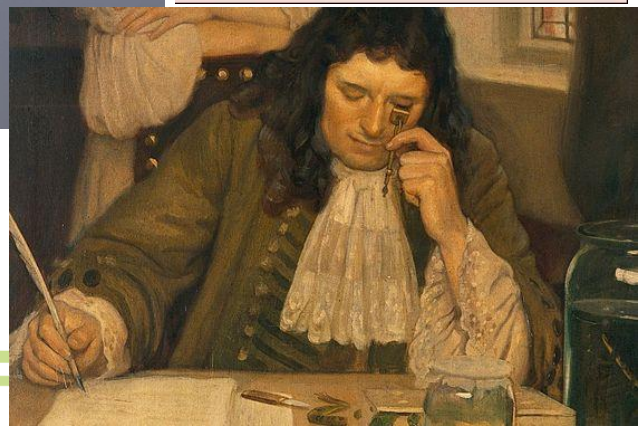
The existence of microrganisms was recognised in the 17th century


Antonie van Leeuwenhoek (1632–1723)

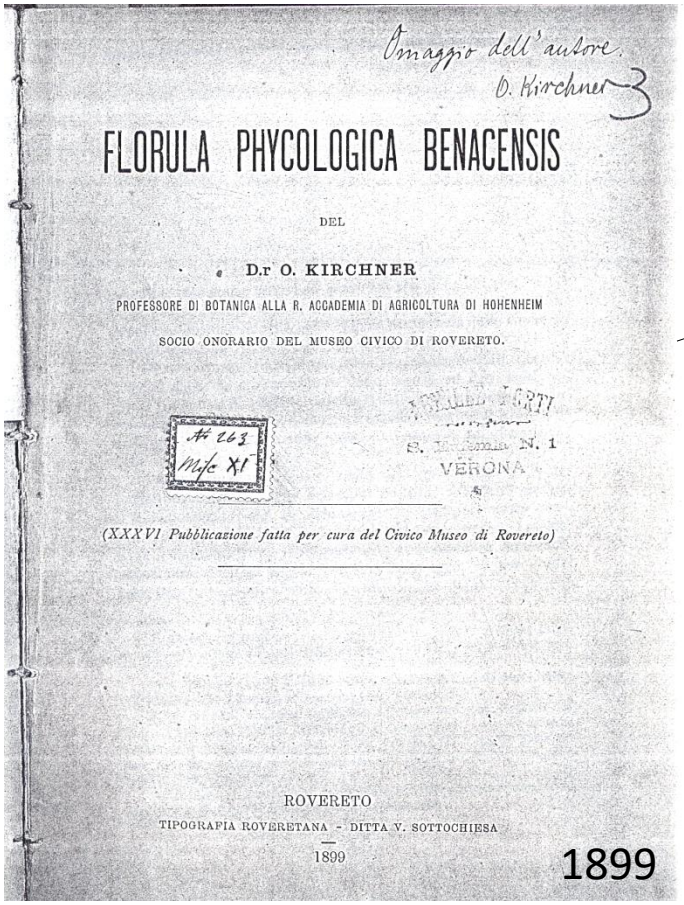








Riconosciuto come il primo:
- Microscopista
- Microbiologo
- Scopritore di protisti (“animalculi”)


Omaggio dell'autore.
O. Kirchner

FLORULA PHYCOLOGICA BENACENSIS

DEL

D.r O. KIRCHNER

PROFESSORE DI BOTANICA ALLA R. ACCADEMIA DI AGRICOLTURA DI HOHENHEIM

SOCIO ONORARIO DEL MUSEO CIVICO DI ROVERETO.

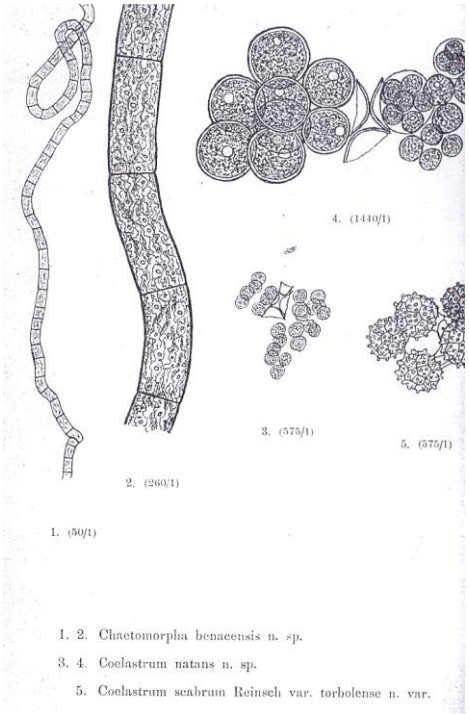
A. 263
Mfc XI

S. [illegible] N. 1
VERONA

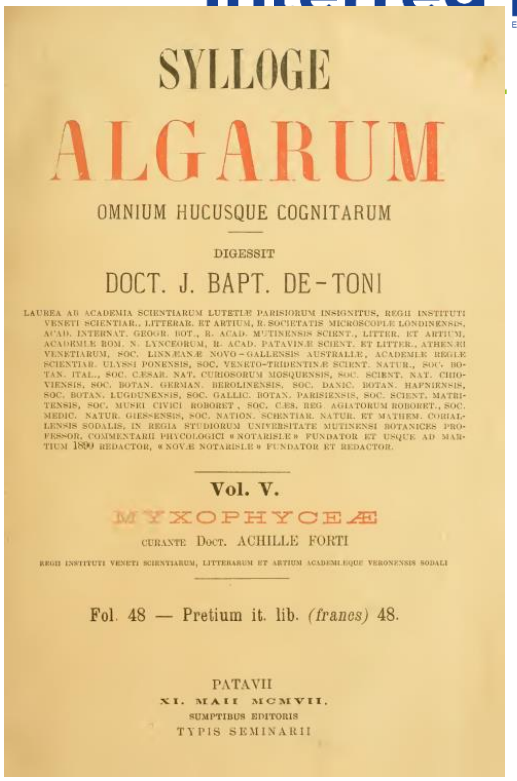
(XXXVI Pubblicazione fatta per cura del Civico Museo di Rovereto)

ROVERETO
TIPOGRAFIA ROVERETANA - DITTA V. SOTTOCHIESA
1899

1899


4. (1440/1)
3. (575/1)
5. (575/1)
2. (260/1)
1. (50/1)

1. 2. Chaetomorpha benacensis n. sp.
3. 4. Coelastrum natans n. sp.
5. Coelastrum scabrum Reinsch var. torbolense n. var.


SYLLOGE
ALGARUM
OMNIUM HUCUSQUE COGNITARUM
DIGESSIT
DOCT. J. BAPT. DE-TONI

LAUREA AB ACADEMIA SCIENTIARUM LUTETIÆ PARISIORUM INSIGNITUS, REGII INSTITUTI VENETI SCIENTIAR., LITTERAR. ET ARTIUM, R. SOCIETATIS MICROSCOPICÆ LONDINENSIS, ACAD. INTERNAT. GEOGR. BOT., R. ACAD. MUTINENSIS SCIENT., LITTER. ET ARTIUM, ACADEMIÆ ROM. S. LYNCEORUM, R. ACAD. PATAVINÆ SCIENT. ET LITTER., ATHENÆI VENETIARUM, SOC. LINNEANÆ NOVO-GALLENSIS AUSTRALIÆ, ACADEMIÆ REGIÆ SCIENTIAR. ULYSSI PONENSIS, SOC. VENETO-TRIDENTINÆ SCIENT. NATUR., SOC. BOTAN. ITAL., SOC. CÆSAR. NAT. CURIOSORUM MOSQUENSIS, SOC. SCIENT. NAT. CHIOVIENSIS, SOC. BOTAN. GERMAN. BEROLINENSIS, SOC. DANIC. BOTAN. HAFNIENSIS, SOC. BOTAN. LUGDUNENSIS, SOC. GALLIC. BOTAN. PARISIENSIS, SOC. SCIENT. MATRITENSIS, SOC. MUSEI CIVICI ROBORET., SOC. CÆS. REG. AGIATORUM ROBORET., SOC. MEDIC. NATUR. GIESSENSIS, SOC. NATION. SCIENTIAR. NATUR. ET MATHEM. COGNALENSIS SODALIS, IN REGIA STUDIORUM UNIVERSITATE MUTINENSI BOTANICES PROFESSOR, COMMENTARII PHYCOLOGICI « NOTARISIÆ » FUNDATOR ET USQUE AD MARTIUM 1899 REDACTOR, « NOVÆ NOTARISIÆ » FUNDATOR ET REDACTOR.

Vol. V.
MYXOPHYCEÆ
CURANTE DOCT. ACHILLE FORTI
REGII INSTITUTI VENETI SCIENTIARUM, LITTERARUM ET ARTIUM ACADEMIÆQUE VERONENSIS SODALI

Fol. 48 — Pretium it. lib. (franes) 48.

PATAVII
XI. MAII MCMVII.
SUMPTIBUS EDITORIS
TYPIS SEMINARII

1907

9. **Anabæna Lemmermanni** Richt. in Lemm. Phytopl. Sächs. Teiche pag. 38 in Plöner Berichte t. VII et in Beitr. z. Kenntn. d. Planktonalgen XV, in Plöner Berichte X, p. 153, *Anabæna Flos-aquæ* Klebahn in Flora 1895, p. 27, tab. IV, fig. 21-22. — Strato subsphærico vel elongato, ad 150 μ. diam.; trichomatibus e plurimis inter se intricatis, radiatim dispositis, arcuatis; articulis subsphæricis vel ellipsoideis, rarius diametro brevioribus, latere externo convexis, interno fere rectis, 5,5-7 μ. crassis, 5-8 μ. longis; sporis cylindricis, leviter lunato-curvatis, apicibus rotundatis, 8-11 μ. diam., 19-31 μ. longis, e plurimis plerumque prope heterocystas sitis. 1021

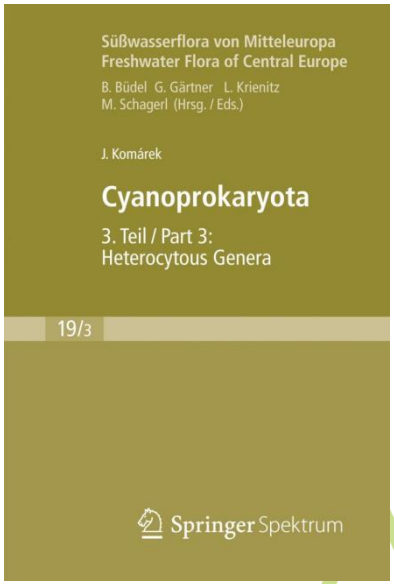
Hab. in lacubus Saxoniæ et Holsatiæ (LEMMERMANN), Zwischenahner Meer (LEMMERMANN) et in lacu Huro ins. Chatham oceani Pacifici (SCHAUINSLAND).

10. **Anabæna circinalis** Rab. Alg. exs. n. 209! (nec n. 1674, 2064), 1022

Pavesi, P. 1877. *Intorno all'esistenza della fauna pelagica o d'alto lago anche in Italia.*
Bull. Entomol ., 9: 293-298.
Pavesi, P. 1879a. *Nuova serie di ricerche sulla fauna pelagica dei laghi italiani. Rendic. R. Ist. Lomb ., 12 (16): 688-707.*
Pavesi, P. 1879b. *Ulteriori studi sulla fauna pelagica dei laghi italiani. Rendic. R. Ist. Lomb ., 12 (11-22): 474-483.*
Pavesi, P. 1883. *Altra serie di ricerche e studi sulla fauna pelagica dei laghi italiani. Atti Soc. Ven. Trent. Sc. Nat ., 8: 340-4*
***A. Garbini*, 1899**. Alghe neritiche del Lago di Garda. Nuova Notarisia, X
(...)

2nd half 1600s - 1800s

2019



Interreg Alpine Space Eco-AlpsWater

Microscopical identification of species

The modern microscopical classification of species is based on the identification of discriminant morphological and morphometrical characters

Aphanizomenon

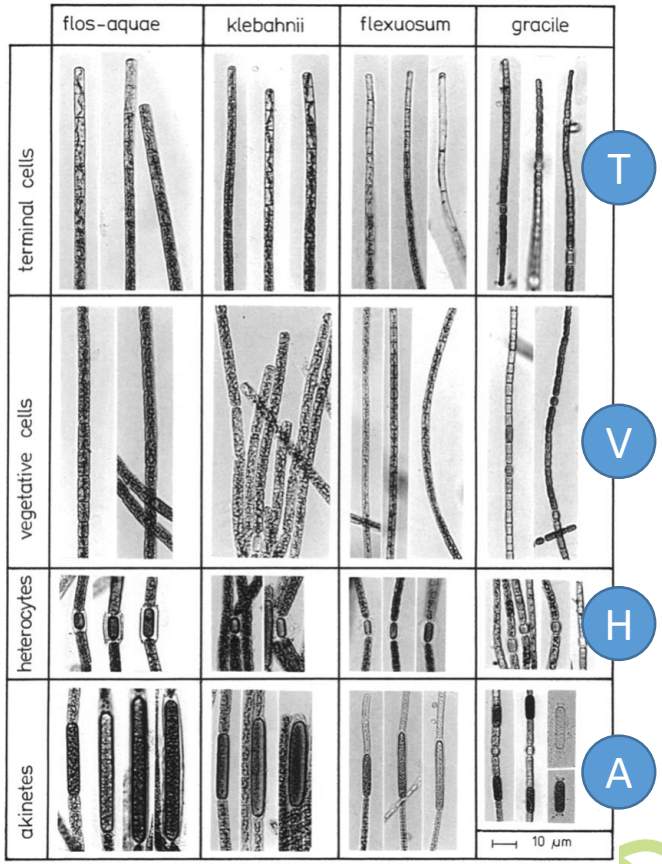


Fig. 3. Terminal cells, vegetative cells, heterocysts and akinetes of the four *Aphanizomenon* taxa investigated from Czechoslovakian biotopes

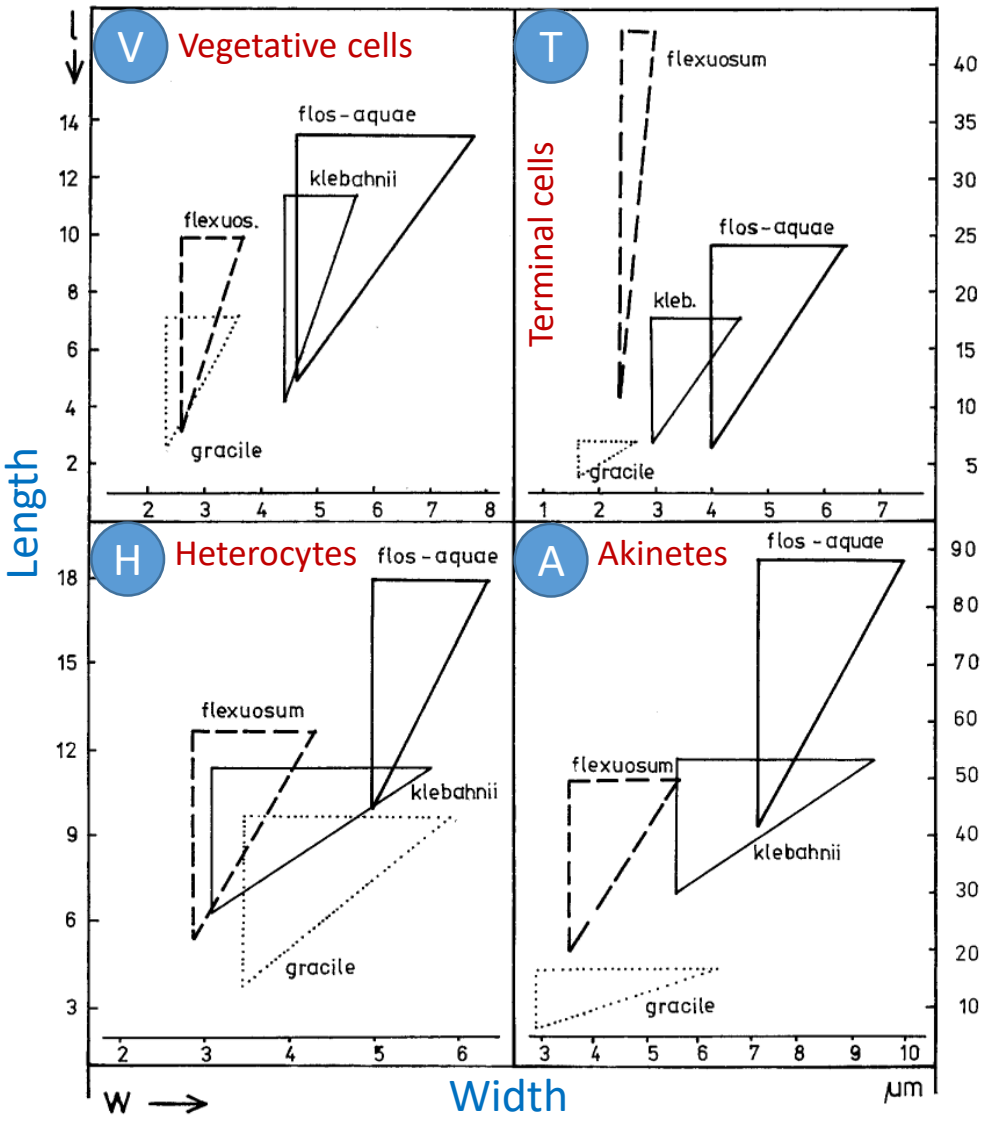
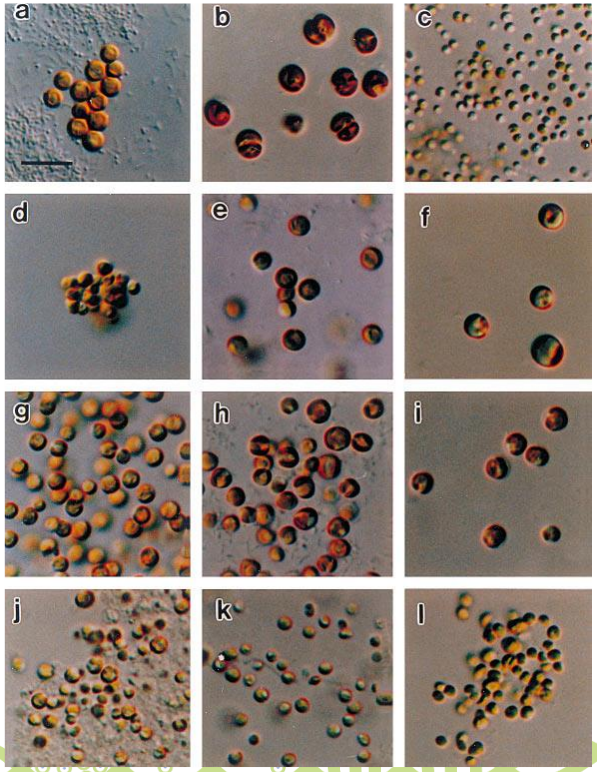


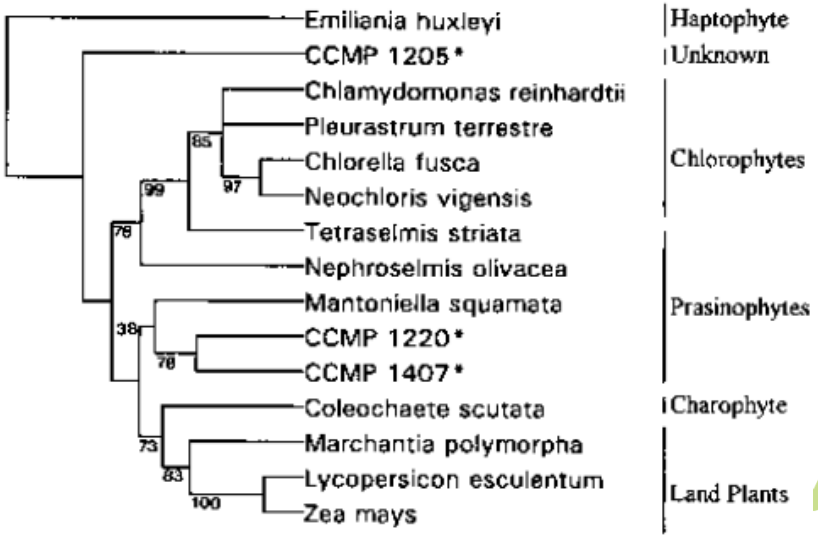
Fig. 4. Comparison of length (l): width (w) ratios of vegetative cells (V), terminal cells (T), heterocysts (H) and akinetes (A) of the four *Aphanizomenon* taxa investigated

A major drawback of classifications based on the numerical analysis of characters (Phenetic) is that distantly related species can share morphological (and physiological) attributes by **convergent evolution**, that is the independent evolution of analogous characters in different lineages

Analogous characters



In the oceans, the `little brown balls' have evolved independently at least in three distinct eukaryotic lineages (heterokont algae, haptophyte algae, and green algae),



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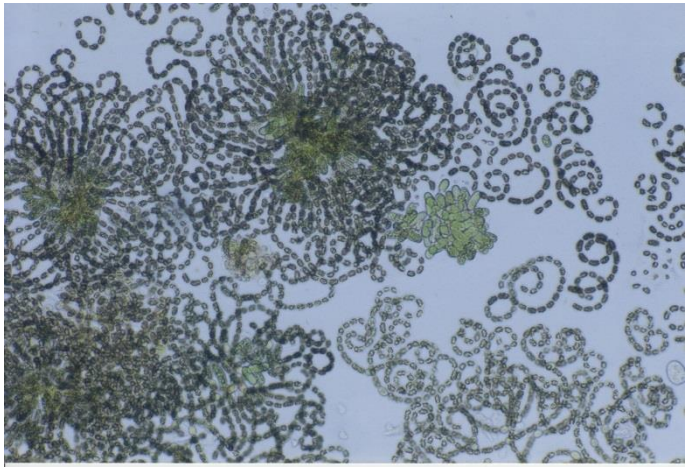
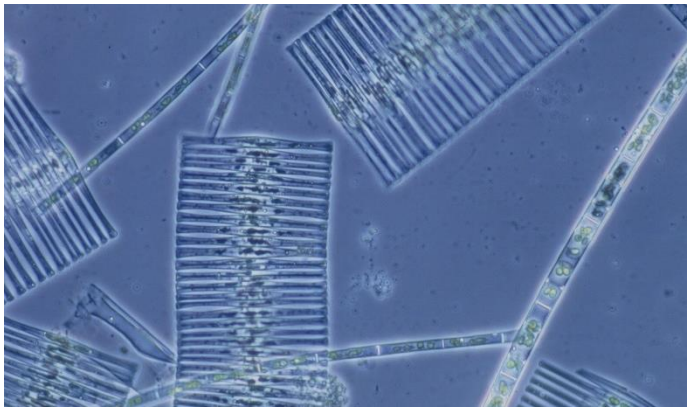
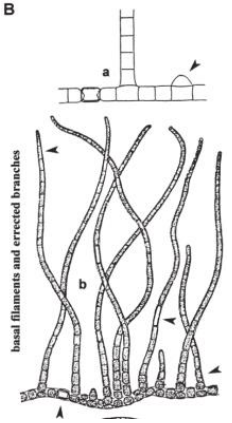
Microscopic examination of algal samples based on the identification of diacritical characters – DRAWBACKS!



1a.	Heterocytes[2] and/or akinetes never occur in trichomes: order Oscillatoriales.....2
1b.	Heterocytes and/or akinetes develop commonly or occasionally in trichomes (if heterocytes are lacking, trichomes are morphologically complex with true branching or with akinetes).....35
2a.	Trichomes (without sheaths) very narrow and cylindrical; ≤ 3 µm wide (rarely up to 6 µm); cells sometimes with separated centroplasma and chromatoplasma (parietal arrangement of thylakoids).....3
2b.	Trichomes broader; width > 3 µm, usually 4–16 µm (rarely to 60 µm); trichomes cylindrical to moniliform; cell content homogeneous or variably structured (thylakoids arranged radially or irregular).....16
3a.	Trichomes without sheaths or within simple, thin sheaths (when present, always one trichome per sheath), solitary or in mats; trichomes (or filaments) isopolar (both poles with same morphology) with exception (see Figs. 11b and 14): family, Pseudanabaenaceae.....4
3b.	Sheaths wide, containing one or two or more trichomes, at least in a part of a filament; filaments mainly heteropolar: family Schizotrichaceae.....15
4a.	Trichomes without individual sheaths, but may possess wide or diffuse, mucilaginous envelopes.....5
4b.	Trichomes with distinct, thin, fine or firm sheaths.....11
5a.	Trichomes straight, wavy, or irregularly coiled.....6
5b.	Trichomes in regular, screwlike coils.....10



- Overlapping morphological features
- Requires great experience, and ability to make use of manuals and «sparse» literature
- Plasticity of phenotypic characteristics that can vary with environmental conditions
- Ineffective for identification of the smallest species
- Often, results obtained by different observers are not comparable



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European Regional Development Fund

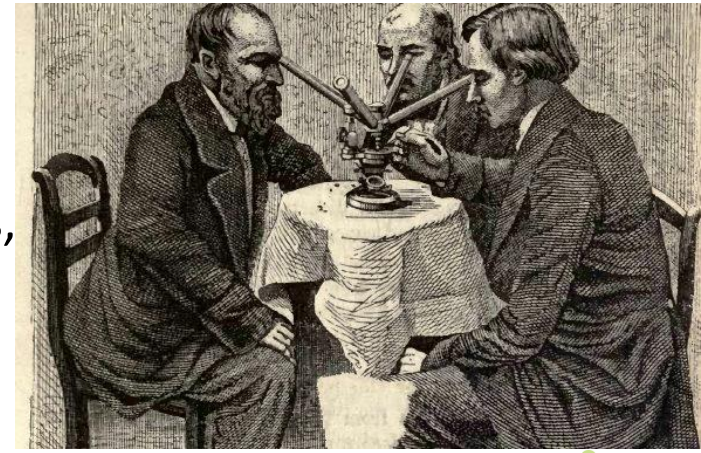
Polyphasic identification of cyanobacterial isolates from Australia

WATER RESEARCH 59 (2014) 248–261

Elvina Lee [a], *Una M. Ryan* [a], *Paul Monis* [b], *Glenn B. McGregor* [c], *Andrew Bath* [d], *Cameron Gordon* [d], *Andrea Paparini* [a,*]

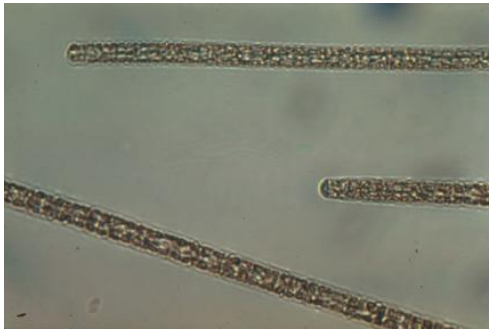
Lee et al. (2014) compared the morphological and molecular characterisations of cyanobacterial isolates (n = 39) collected from various freshwater sites in Australia.

- Overall, only 46% of the isolates (18/39) were morphologically identified to species level by at least one taxonomist
- Of all the isolates analysed by both taxonomists, species identifications were in complete agreement for three isolates (18%, 3/17)
- Genus identifications were in agreement for six isolates (35%, 6/17)



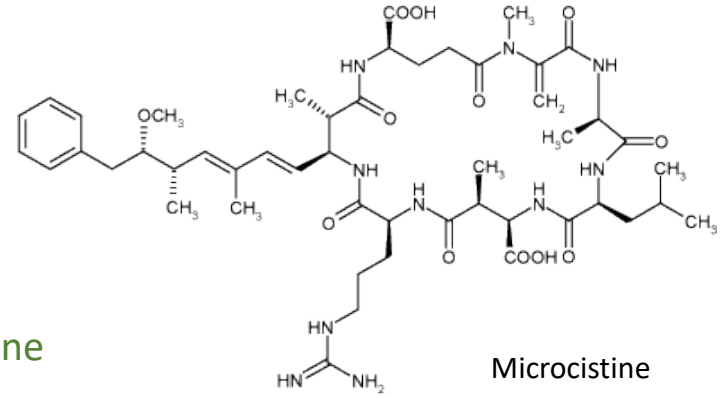
http://www.maastrichtsts.nl/advancing-microscopy/

Rilevanza di una corretta identificazione

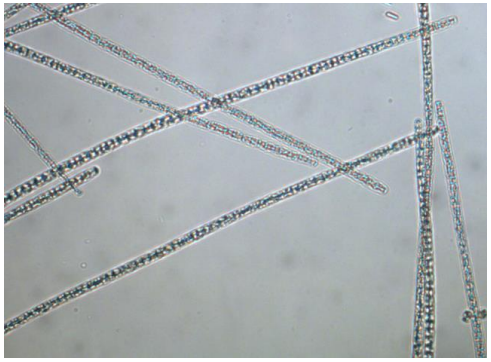


Planktothrix rubescens

Laghi profondi
Produce epatotossine (microcistine

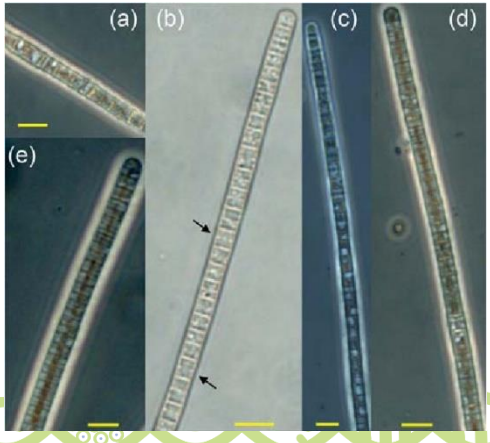


Microcistine



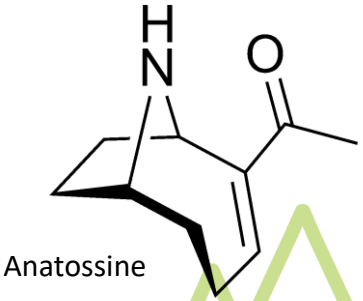
Planktothrix agardhii

Laghi di bassa profondità, torbidi
Produce epatotossine (microcistine



Tychonema bourrellyi

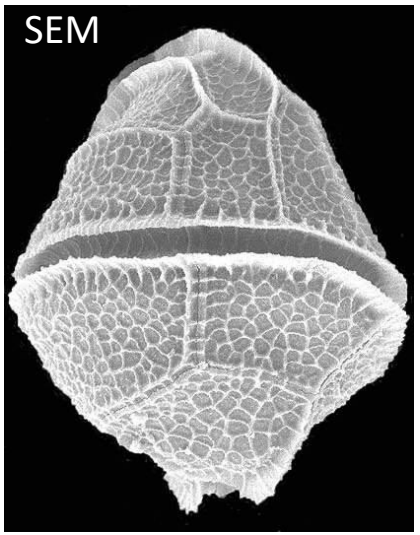
Laghi generalmente profondi e media profondità
Produce neurotossine (anatossine)

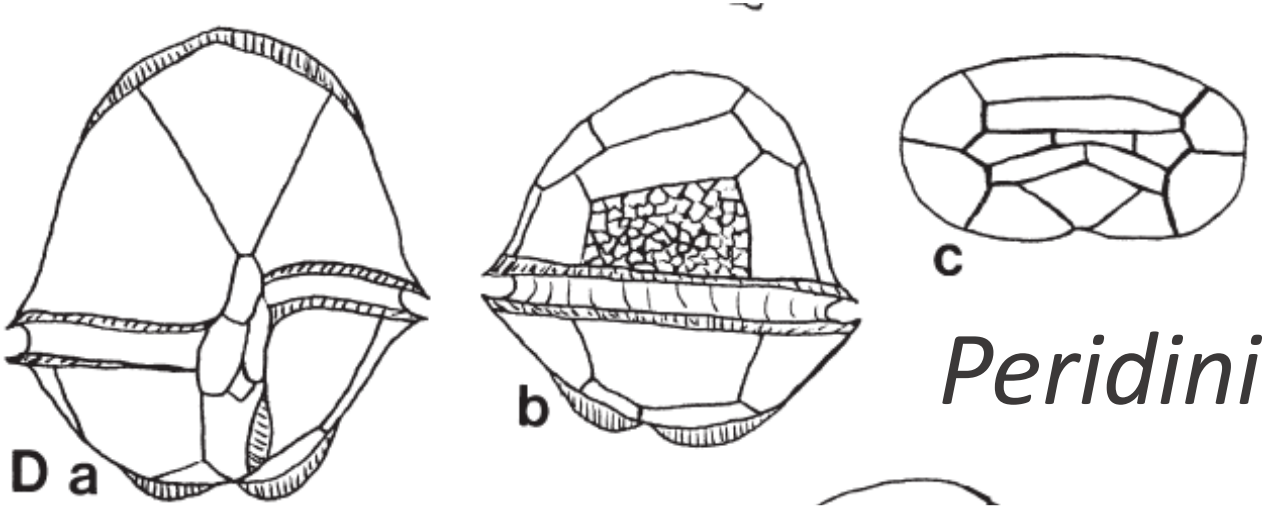


Anatossine

Rilevanza di una corretta identificazione

Dipendenza dalle caratteristiche del campione





Peridinium willei

Great uncertainty in the taxonomic attribution of several algal groups by light microscopy, especially in fixed samples

Is it really conceivable to think that further progress can be made in the study of biodiversity, ecology and monitoring using technologies defined over 300 years ago and adopted in modern ecological studies over the last century?

PART C - Project description

C.1 Project relevance

What are the common territorial challenges and/or joint assets that will be tackled by the project?

The assessment of the ecological status in EU is facing serious challenges, due to the limits of the traditional time-consuming and expensive classical identification of aquatic biota. Considering the huge advance in science, innovative next-generation sequencing monitoring tools are available now to complement traditional monitoring approaches and to identify efficient action plans for the recovery of water resources.

La valutazione dello stato ecologico nell'UE si trova di fronte a gravi sfide, a causa dei limiti della tradizionale costosa e dispendiosa [*incerta - incompleta*] identificazione del biota acquatico. Considerando l'enorme progresso scientifico, sono ora disponibili innovativi strumenti di monitoraggio del sequenziamento di prossima generazione in grado di integrare i tradizionali approcci di monitoraggio, favorendo una migliore identificazione dei piani d'azione efficaci per il recupero delle risorse idriche.